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(54) Title: **MULTIPLEX ASSAY DETECTION OF PATHOGENIC ORGANISMS**

(57) Abstract: The present invention is directed to a method for identification of a pathogenic organism from a predetermined group of pathogens, comprising (i) isolating a clinical sample containing at least partially purified nucleic acid, (ii) subjecting at least a first aliquot of said clinical specimen to at least one amplification and detection reaction in one reaction vessel comprising (iia) an amplification step using at least a first set of amplification primers capable of amplifying a preselected nucleic acid sequence region from several or all members of said predetermined group of pathogens, (iib) a detection step using at least 2, 3 or multiple hybridization reagents, said reagents together being capable of specifically detecting a pre-selected nucleic acid sequence region from all members of said group of pathogens, said detection step (iib) comprising steps monitoring hybridization of each of said hybridization reagents at a pre-selected temperature, said hybridization being indicative for at least the genus of said pathogen present in the sample, and monitoring temperature dependence of hybridization, said temperature dependence being indicative for at least the species of said pathogen, and (iii) determining whether said amplification and detection reaction is indicative for the presence of a specific member of said pre-selected group of pathogens.